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Simple Procedure for Jugular Vein Cannulation of Rats

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Summary: Jugular vein cannulation of rats is a method widely used in biochemical toxicology studies to allow intravenous administration of toxicants and/or temporal collection of blood samples. Methods typically used for this surgery produce tissue trauma, may partially interrupt the systemic circulation, and require a fair amount of practice by the technician in order to achieve proficiency. The method described here is simple to perform, produces minimal trauma to the rat resulting in rapid recovery, and allows an intact circulation to be maintained. Following limited isolation of the vein by blunt dissection, a 22-gauge catheter is threaded into the bore of an 18-gauge needle, which, in turn, is inserted through the back of the neck and into the surgical field. The needle shaft is removed and the catheter inserted into the exposed jugular vein using another 18-gauge needle that has been longitudinally sectioned to make a trough to carry the catheter. Once the catheter is threaded into the vein, the needle shaft is removed and the vessel sealed with a silicone rubber patch and surgical glue. The insertion site at the back of the neck is similarly sealed and the ventral incision closed with surgical staples. The catheter is coiled and placed beneath a wrap of tape or a Velcro jacket. Patency can be maintained for more than a week using sterile saline flushes and heparin blocks. **Key Words:** Cannulation—Rat jugular vein—Serial blood sampling—22-gauge catheter—Toxicokinetic methods—Intravenous administration—Simple surgical procedure.

Jugular vein cannulation of small animals is a common method for blood collection or administering substances intravenously. The surgical cut down to the external jugular vein and catheter implantation can be accomplished using a variety of methods and materials. A common method for laboratory rats has been previously described (1), and citations for numerous other methods have been compiled (2). These methods of cannulation often require complete isolation of the jugular vein and as a result may produce considerable surgical trauma, post-operative discomfort and a prolonged recovery. Additionally, the conventional method of cannulation generally

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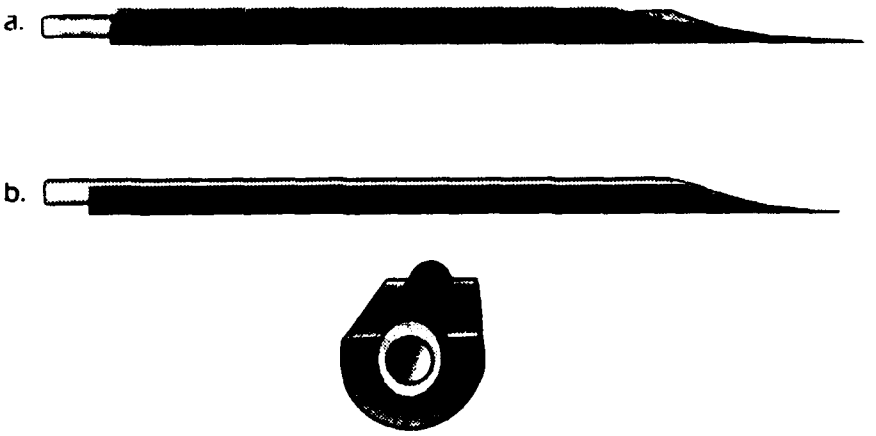

FIG. 1. Diagram of needle/catheter assemblies. **a:** Assembly for insertion of catheter through the neck. **b:** Assembly for insertion of catheter into jugular vein.

requires substantial training and practice in order for the surgical procedure to be consistently successful.

The procedure we describe is a modification of a method previously developed (3) and consists of "limited" isolation and cannulation of the jugular vein using an 18-gauge needle. We have found this method much less traumatic and quicker to perform, and it can be learned by untrained personnel with minimal expenditure of time and resources.

EXPERIMENTAL PROCEDURE

Ketamine HCl solution (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, IA) and xylazine (Rompun, Mobay Corp., Animal Health Division, Shawnee, KA) are used for anesthesia. Sterile saline is prepared for use as catheter flush by filtering the solution through a 0.2-μ filter.

Two hypodermic needles (18-gauge, length 1.5 in, Becton Dickinson and Co., Rutherford, NJ) are prepared for use by removing the syringe hubs. This is easily done by cutting the needle shaft with a file and then breaking the needle with a bending motion. One needle will be used to pass the catheter through the neck, dorsal to ventral orientation (Fig. 1a). The second of the two needles is cut in half longitudinally forming a U-shaped trough that can be pressed onto a 22-gauge catheter (Fig. 1b). Cutting the needle longitudinally is easily accomplished using a belt sander. The needle is sanded prior to removing the hub so that the hub serves as a handle which is held with a pair of pliers. The sandpaper used for preparation can be of any type, but a fine grit, no. 100 or finer, is most satisfactory. This half needle is used to insert the catheter into the vein, as described below. Both types of needle shafts are included with the surgical pack which is autoclaved and maintained sterile until use.

Rats are anesthetized by i.p. injection (25-gauge needle) of a solution of ketamine:xylazine prepared in a 7:3 ratio; the initial concentration of the ketamine

and xylazine [illegible]00 mg/ml and 20 mg/ml, respectively. The dose of anesthetic is administered [illegible]ntally in 0.1 ml volumes, up to a concentration of 1.0 ml/kg. Unconsciou[illegible] should be achieved in ~5 min, at which time the fur is shaved from the neck region and the skin cleaned and sterilized with alcohol swabs or a solution of iodine. The animal is placed on its back on a surgery board, head toward the operator, and the forelimbs taped to the board using masking tape. Prior to making the incision, the neck is wiped thoroughly with an alcohol pad (Triad Medical, Inc., Franklin, WI), and the rat's eyes are protected from drying by applying Muro 128 ophthalmic ointment (Bausch and Lomb Pharmaceuticals, Inc., Clearwater, FL). All surgical instruments (two straight and one curved hemostats, one curved forceps, and one scalpel) are sterilized by autoclaving prior to beginning the surgery. An aseptic field is maintained during surgery by placing surgical instruments in a germicidal solution (Amerse Hospital Instrumen[illegible]rmicide, Calgon Vestal Laboratories, St. Louis, MO) during the surgical proc[illegible].

Using a scalpel, a longitudinal incision is made ~1 cm to the right of midline (Fig. 2a), beginning at the pectoralis mus[illegible]e and extending 2.5 cm toward the head. The incision is made with one motion, and the underlying tissue and blood vessels remain untouched. The jugular vein, which is directly beneath the subcutaneous tissue, is exposed by holding the skin with hemostats in one hand and picking the tissue from the vessel with forceps in the other hand. Care should be taken to minimize trauma to the jugular vein, which will cause the vessel to vasoconstrict. The diameter of the carefully isolated jugular vein is ~2 mm. Applying pressure to the vessel where it passes beneath the pectoralis muscle will obstruct blood flow, causing the vein to dilate, and thereby make insertion of the needle easier.

A loose ligature (2-0 silk) is placed beneath the jugular vein (Fig. 2a), cephalad to the site of insertion of the catheter. Placement is most easily accomplished by blunt dissection beneath the vessel, being careful not to traumatize the vein. Once in place, lifting up on the ligature serves to occlude the flow of blood from the head and therefore minimizes the loss of blood once the vessel is penetrated by the half needle. Additionally, the ligature is an anchor which provides resistance needed when the catheter is inserted.

The catheter (22-gauge cutdown catheter, Deseret, Sandy, UT) is prepared by connecting the catheter hub to a 1-ml syringe filled with sterile saline. The tip of the catheter is cut with a scalpel to produce a slight bevel at approximately a 15° angle. Although a sharper bevel will make it easier to insert the catheter into the vein, it also increases the chance of perforating the vessel. A sharp bevel may also prevent withdrawal of blood samples because the catheter opening adheres to the vessel wall when suction is applied through the syringe. After cutting the bevel, the catheter is inserted into the cut/broken end of the 18-gauge needle. Sterile saline is flushed into the catheter to remove the air, and the 18-gauge needle, catheter inside, is carefully passed through the back of the neck into the area of the ventral incision (Fig. 2b); care is taken to avoid puncturing vascular beds in the neck. Once through the neck, the needle is removed from the shaft of the catheter and the half needle pressed onto the end of the catheter shaft so that the bevels of the needle and catheter are in approximately the same plane. The needle/catheter assembly is passed through a sterile piece (1 cm in diameter) of silicone rubber sheeting (Silastic Brand sheeting no. 501-1, Dow

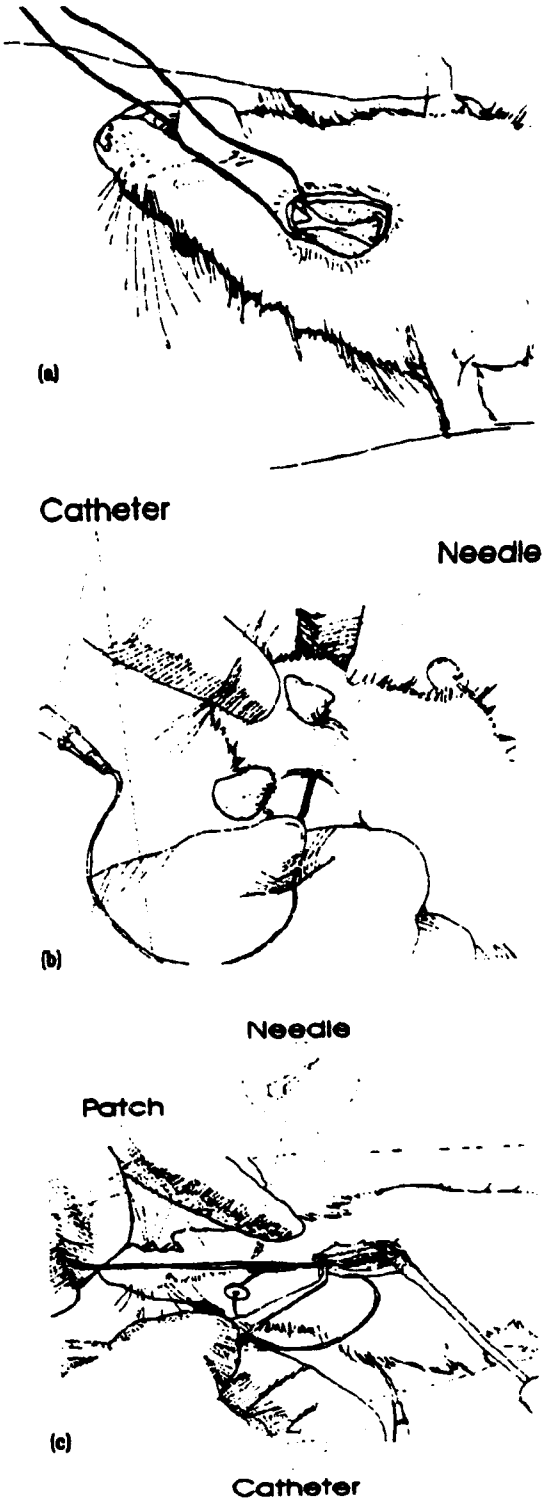

FIG. 2. Sketch of sequential steps in the cannulation of the external jugular vein. **a:** Jugular vein exposed and ligature placed cephalad to the site of insertion. **b:** Needle/catheter assembly inserted through the back of the neck. **c:** Insertion of the half-needle/catheter assembly into the jugular vein.

Corning Corp., Medical Products, Midland, MI) and the silicone sheet slid on to the shaft of the catheter. The rounded patches of sheeting are autoclaved prior to use.

The catheter/needle assembly is held with the thumb and index finger of one hand with the bevels up. Insertion of the needle (depicted in Fig. 2c) should be made at a point where the greatest chance of success exists; this point is most often in the segment of the vein between the bifurcation of the brachial vein and pectoralis muscle. A good bit of force may be required to penetrate the vessel, and resistance to the forward thrust is provided by holding the ligature in the opposite hand. Because the diameter of the needle is sometimes larger than the vein, the needle must be inserted far enough to cover the bevel of the needle and allow the catheter to be inserted into the lumen of the vessel, while at the same time being careful not to penetrate the opposite side of the vessel. Once inserted into the lumen of the vessel, carefully rest the catheter on the surgical board and, holding the needle stationary with fingers or forceps, slide the catheter into the vein far enough to prevent its accidental escape from the vessel (~1–2 cm). Hold the catheter stationary as the needle is retracted from the vein and catheter and removed from the surgical field. [Note: As this method has been used with more frequency within our laboratory, we have found that some technicians prefer to use an intact needle to perforate the vessel, rather than the half needle assembly. Then using forceps to hold the naked catheter, it is inserted into the vein, (two steps instead of one). Using this approach allows more blood to escape into the surgical field but does not appear to hinder the overall technique.] Using either approach, the catheter can then be slid the remaining distance necessary to reach the right atrium (total insertion of ~7.5 cm), which can be determined when resistance is met, and then withdrawn slightly.

It is not uncommon to meet resistance within 2–3 cm of the insertion site. If this happens the catheter has to be moved to different positions until it passes the obstruction. Various techniques can be used to help the insertion, such as raising the chest cavity off the surgery board, holding the wall of the jugular vein with forceps, and/or injecting small amounts of saline to lubricate and open the vessel.

Once the catheter is in place, draw back slightly on the syringe to see if blood flows freely from the vein. The insertion site on the vessel surface is made dry by swabbing with a cotton tip applicator. If the ligature has been continually held, little or no blood should have escaped from the insertion site. The catheter is then anchored in position by sliding the patch of silicone sheeting over the insertion site. The sheeting is anchored in place by applying surgical adhesive (Vetbond Tissue Adhesive, No. 1469, 3M Animal Care Products, St. Paul, MN) to its surface and the surrounding tissue. The effect of applying the sheeting is like forming a patch or "scab" over the insertion site, which allows the lumen of the vessel to remain patent. Drying time for the adhesive is typically 1–2 min, at which time the loose ligature can be removed and blood flow restored to the jugular vein.

Patency is verified by drawing back on the syringe and observing whether blood flows into the catheter. Infuse the catheter with a sterile saline flush, ~0.5 ml, and then quickly change the syringe to one containing 100% heparin. A volume of 0.08 ml exactly fills the catheter without reaching the animal. Remove the syringe and replace the catheter cap. Care should be taken to minimize the amount of heparin injected into the animal to decrease the chance of hemorrhage following surgery. To

minimize blood loss and possible hemorrhage, the surgical field may be cauterized using Silver Nitrate Applicators (Graham-Field Surgical Company, Inc., New Hyde Park, NY).

Close the ventral surgical opening with sutures or stainless steel suture clips (9 mm, Clay Adams, Division of Becton, Dickinson and Co.). The exteriorized catheter is coiled and held in place with tape wrapped around the chest of the animal or by using a Velcro jacket. Following the surgery, watch the animal for signs of hemorrhaging. The rat should recover from the anesthesia within 30 min.

DISCUSSION

This method was developed out of attempts to perform cannulations using a previously described procedure (3) which made use of silicone tubing glued to a combination of 20- and 23-gauge needles. Preparation of the silicone tubing-needles assembly was somewhat labor intensive compared to the method described here. The silicone tubing offered the advantage of being less thrombogenic but, being made of very pliable rubber, was quite difficult to insert into the vein, and a considerable amount of training and practice was necessary to achieve success with this technique. The procedure described here is simple to perform, requires little training and practice, causes a minimal amount of trauma, and allows the circulation to remain intact.

Since the technique developed in this study does not disrupt the circulation, it is possible to cannulate both the right and left external jugular veins for purposes of administration and blood collection. In conducting toxicokinetic or metabolic studies, it is generally considered poor practice to perform both administration and blood collection using the same catheter. Separate vessels are used for administration of toxicants and removal of blood samples. Use of a single, relatively less traumatic method to cannulate two vessels should improve the overall success of the surgeries and the survival of the animals. Examples of vessels that have been used for sampling, while the external jugular vein is used for administration, include the contralateral carotid artery (4), the aorta (5), and the femoral vein (6). Alternatively, the jugular vein has been used for blood collection and the tail or penile vein (7) used for toxicant administration. A method for placing two catheters in a single jugular vein in mice for purposes of simultaneous infusion and blood sampling was also recently described (8).

Our experience has been that catheters prepared as described above can be routinely inserted in ~20 min, start to finish, and will remain patent for >1 week. The length of time that patency is maintained is improved when the catheter is flushed (sterile saline) at least once each day and the 0.08-ml heparin block replaced. If daily flushing is not performed, the catheters become occluded within 2–3 days. We found heparin alone to be the most satisfactory anticoagulant for maintaining patency, as long as it is delivered in a volume limited to the capacity of the catheter. Other authors have reported using a catheter block consisting of a 9:1 mix of glycerine/heparin (9). The increased viscosity of the solution acts to prevent mixing of blood with the blocking solution. However, the increased viscosity may also have the effect of preventing complete removal of the block, and blood samples may contain small

amounts of glycerine. Residual glycerine may also be washed into the systemic circulation during flushing of the catheter with saline.

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REFERENCES

1. Waynforth HB. *Experimental and surgical technique in the rat.* London: Academic Press, 1980:50–2.
2. Petty C. *Research techniques in the rat.* Springfield, IL: Charles C. Thomas Pub., 1982:91.
3. Bakar SK, Niazi S. Simple reliable method for chronic cannulation of the jugular vein for pharmacokinetic studies in rats. *J Pharm Sci* 1983;72:1027–9.
4. Hirayama H, Morgado J, Gasinska I, Pang KS. Estimations of intestinal and liver first-pass metabolism *in vivo:* studies on gentisamide conjugation in the rat. *Drug Metab Dispos* 1990;18:588–94.
5. Mills CO, Freeman JF, Salt PJ, Elias E. Effect of anaesthetic agents on bile flow and biliary excretion of ^{131}I-cholylglycyltyrosine in the rat. *Br J Anaesth* 1989;62:311–5.
6. Millard W, Fox TO, Badger TM, Martin JB. Gonadal steroid modulation of growth hormone secretory patterns in the rat. In: Robbins RJ, Melmed S, eds. *Acromegaly.* New York: Plenum Press, 1987:139–49.
7. Wyman JF, Serve MP, Hobson DW, Lee LH, Uddin DE. Acute toxicity, distribution, and metabolism of 2,4,6-trinitrophenol (picric acid) in Fischer 344 rats. *J Toxicol Environ Health* 1992;37:313–27.
8. Hodge DD, Shalev M. Dual cannulation: a method for continuous intravenous infusion and repeated blood sampling in unrestrained mice. *Lab Anim Sci* 1992;42:320–2.
9. Brown DF, Burr RE. Customized vascular catheters for rodents. *Lab Anim Sci* 1985;35:515–6.